

Chapter 10

Reporting Results

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Reporting Grids

You can use a grid template of circular or rectangular cells to quantitate activity in a regular array such as a dot blot or a slot blot. Using the grid template, you can report quantitative results from the image.

To report the results from the grid template (a grid must be placed over the image):

- ☐ Select **Report Template** from the **Analysis** menu,

Or,

- ☐ Select **REPORT** from the template bar.

The **Grid Report Options** window is displayed (Figure 10-1).

 **Note:** *If more than one template is on the image, the **Template Report** window is displayed. See the section *Using the Template Report Window* (Chapter 9 of the *Getting Started Manual*).*

Using the Grid Report Options Window

The **Grid Report Options** window enables you to report the activity within the cells of the grid.

Using this window, you can report on this activity in one of the following ways:

- **Gross Counts:** The total counts in the cells are reported.
- **Net Counts:** The total counts minus the calculated background for each cell are reported. This option is grayed out when “Background Subtraction” is set to “None”.
- **Gross CPM:** The total counts per minute in the cells are reported.
- **Net CPM:** The total counts per minute minus the calculated background for each cell is reported. This option is grayed out when “Background Subtraction” is set to “None”.
- **Error:** The statistical counting error as a % is reported.
- **Threshold:** The output of each cell is set to “+” or “-”, depending on whether the CPM in the cell is greater than or less than the threshold parameter you indicate (Threshold [CPM]). The output of each cell is set to “?” if the CPM in the cell is within the boundary of the threshold parameter you indicate.

The remaining selections at the **Grid Report Options** window are described below.

When you finish selecting the reporting option(s):

- ☐ Select **OK** to close the window and accept the report options for the grid template.

An example Grid Template Report is displayed in Figure 10-2.

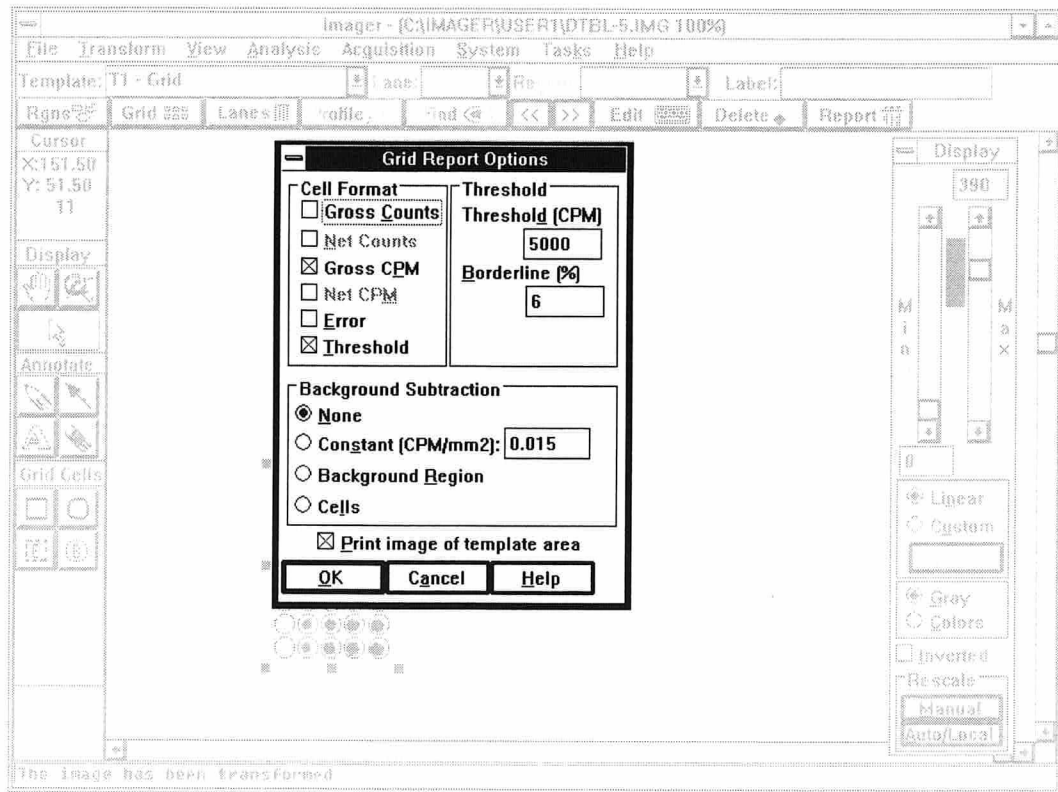


Figure 10–1. Grid Report Options Window.

SELECTION

DESCRIPTION OF USE

Threshold [CPM]

Enter the CPM threshold value. The output of each cell is set to “+” or “–” depending on whether the CPM in the cell is greater than or less than the value you enter.

Borderline [%]

Enter the borderline value, expressed a percentage of the threshold value. CPM values that are within the borderline of the threshold are assigned a “?”.

Background Subtract: None

Indicates that no background subtraction is to be done. When this option is selected, the “Net Counts” and “Net CPM” report options are grayed inactive.

Background Subtract: Constant (CPM/mm²)

Enter the value for the amount of background subtraction in terms of CPM/mm².

SELECTION

DESCRIPTION OF USE

**Background Subtract:
Background Region**

The background subtraction is determined by the CPM/mm² of the background region(s). This selection is grayed inactive if no background regions have been defined for the grid template.

**Background Subtract:
Cells**

The background subtraction is determined by the CPM/mm² of the background cells. This selection is grayed inactive if no background cells have been defined for the grid template.

**Print Image Under
Grid**

Prints the grid over which the selected template is placed when the report is generated. The image is printed at 1/2 of the actual displayed size.

OK

Generates the report

CANCEL

aborts the operation. Closes the window.

HELP

Displays online help about the window.

The Grid Template Report

The Grid Template Report shown in Figure 10–2 has a header on the first four lines. Each header provides identifying information about the image.

The first header contains:

1. **File:** The filename of the image.
2. **Acquired:** The date and time the image was acquired.
3. **Time:** The total elapsed time of the acquisition. The serial number of the instrument's detector may also be displayed.

The second header contains:

4. **Owner/Subject:** The initials of the person responsible for the acquisition. The subject of the image will also be displayed if this information was entered.

The third header contains:

5. **Range:** The minimum and maximum image intensity or counts that are displayed.
6. **Bitmap:** The bitmap can be displayed exponentially or linearly.
7. **Palette:** The palette can be displayed as custom or linearly.
8. **Magn:** The magnification of the bitmap is 50%.

The fourth header contains:

9. **Template Number:** The number of the grid template that is being reported.

The results are reported in an array of rows and columns. The rows are labeled with capital letters, and the columns are labeled with Arabic numerals.

The bitmap of the image is included in the printed copy of the report.

When a "Threshold [CPM]" value is specified at the **Grid Report Options** window, a legend is included in the report. This legend explains the meaning of the characters "–", "?", and "+" that are reported for the output of the cells in the grid.

For example, in Figure 10-3, the following legend is displayed:

Threshold: 5000 $\pm$ 6.00%
 - CPM less than 4700
 ? CPM between 4700 and 5300
 + CPM greater than 5300

A threshold value of 5000 CPM was entered, along with a borderline factor of 6%. Since 6% of 5000 is 300:

- Cells where the CPM count is less than the threshold minus the borderline ($5000-300=4700$) are flagged with a “-”.
- Cells where the CPM count is greater than the threshold plus the borderline ($5000+300=5300$) are flagged with a “+”.
- Cells where the CPM count falls within the borderline of the threshold (4700 and 5300) are flagged with a “?”.

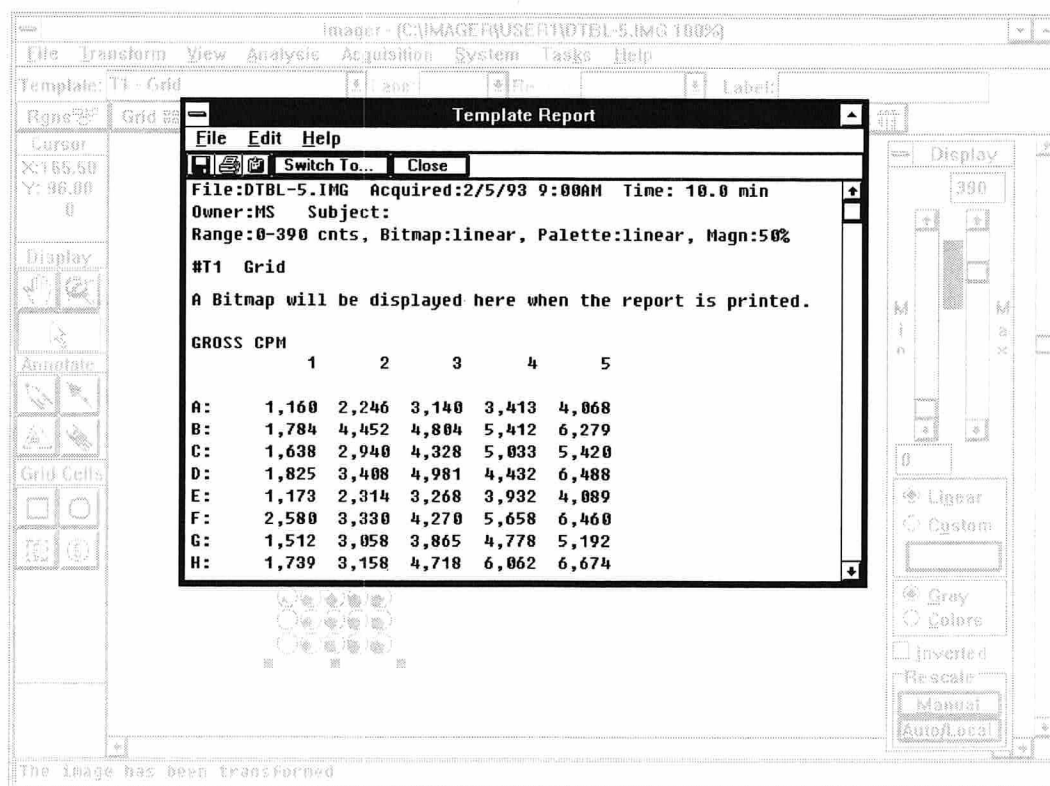


Figure 10-2. Grid Template Report (Gross CPM).

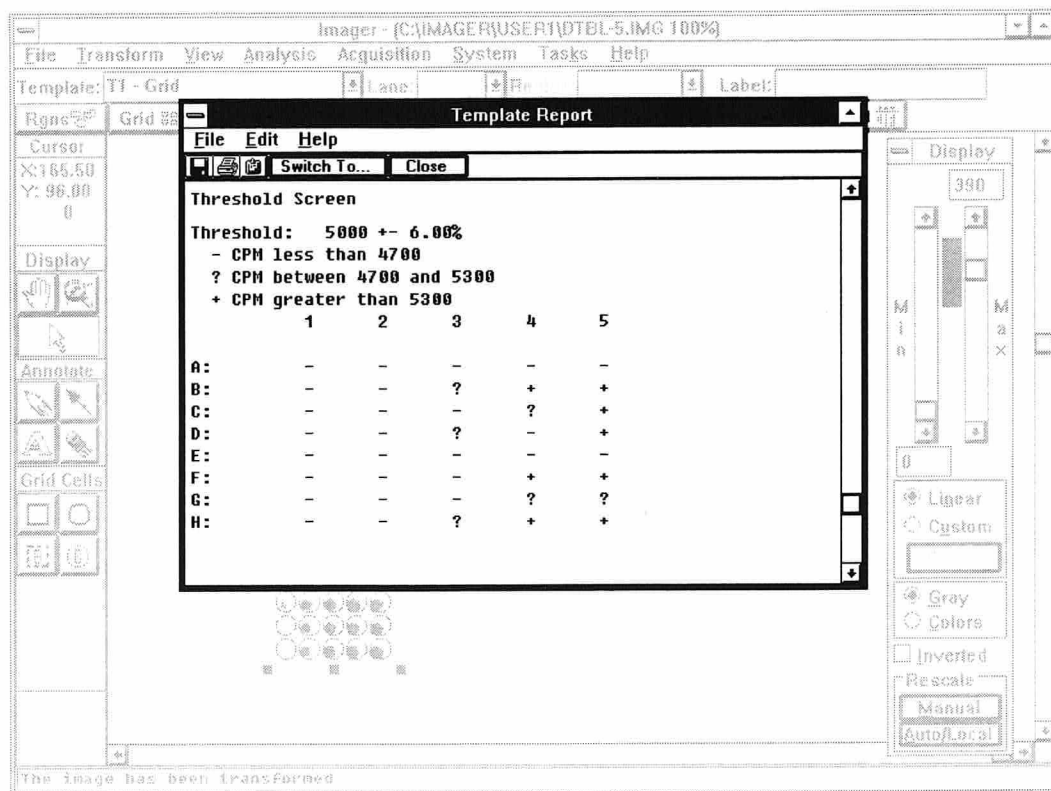


Figure 10-3. Grid Template Report (Threshold Screen).

Reporting Regions

Most samples, including two dimensional TLC plates, two dimensional electrophoresis gels, and tissue sections, can be quantitated by placing regions of interest (ROI) on the image.

You can quantitate ROIs by drawing groups of regions on the image. You can draw regions using a rectangle, ellipse, or an irregular shape. These tools are made available in the tool box of the InstantImager window when a region group is selected.

Each group of regions is identified as T1, T2, etc. Each region within a group is identified with a tag. The region tag can be a number, the counts within the region, or a user-specified label. You can specify the tag type and the position of each tag relative to each region.

You can report the quantitative results obtained from a selected region group.

Using the Group Report Options Window

The **Region Group Report Options** window (Figure 10-4) enables you to report the quantitative results obtained from a group of regions.

Using this window, you can select as few or as many report options as desired. When you select all of the options, you may need to scroll the ensuing reports window horizontally in order to view all of the information.

To display the **Region Group Report Options** window:

- ☐ Select **Report Template** from the **Analysis** menu.

Or,

- ☐ Select **REPORT** from the template bar.



*Note: If more than one template is on the image, the **Template Report** window is displayed. See the section *Using the Template Report Window*, (page GS103).*

Select **OK** to close the window and accept the report options for the selected region group.

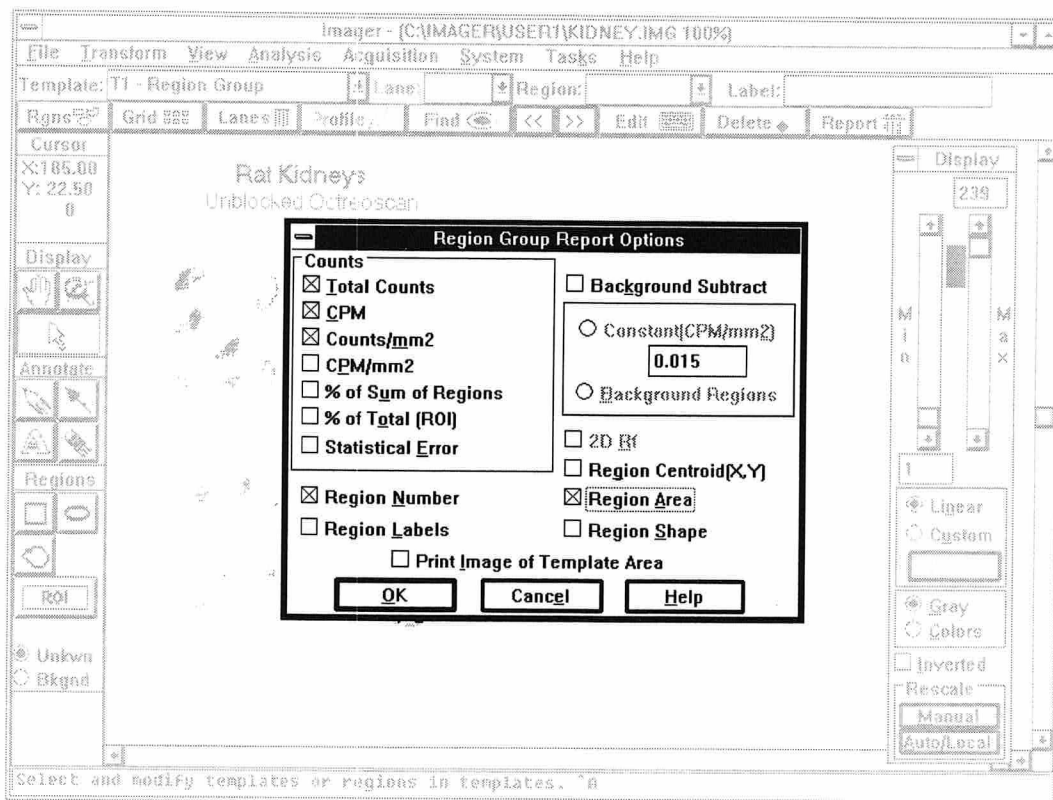


Figure 10-4. Region Group Report Options Window.

The report options at the **Region Group Report Options** window are described below.

<u>SELECTION</u>	<u>DESCRIPTION OF USE</u>
Counts: Total Counts	Reports the sum of the counts in all the pixels within the Regions.
Counts: CPM	Divides the total counts in the regions by the counting time.
Counts/mm²	Divides the total counts in each region by the area of the region in mm ² .
CPM/mm²	Divides the total counts per minute in each region by the area of the region in mm ² .
% of Sum of Regions	Reports the counts in each region as a fraction of the sum of the activity in all the regions of the group.
% of Total (ROI)	Reports the counts as a fraction of the total counts of the entire image or of a ROI if one exists.
Statistical Error	Reports the two sigma statistical counting error in percent $(200/n^{1/2})$, where n is the counts in the region).
Region Number	Reports the region number as listed in the template bar or in the region tags on the image.
Region Labels	Reports the labels (if any) that are used to identify the regions, as listed in the template bar or in the region tags on the image.
Background Subtract	Select this option and then one of the highlighted background subtracting methods below. With both methods the background value in CPM/mm ² will be multiplied by the area of each region. This product will be subtracted from the counts in the region.

SELECTION

DESCRIPTION OF USE

**Background Subtract:
Constant (CPM/mm²)**

Select this option and enter a numerical value in CPM/mm.

**Background Subtract:
Background Regions**

Select this option to determine the background from a region or regions defined as background. The background value will be the sum of the counts in the background regions divided by the total area of the regions. If no background regions exist, the amount of background subtraction will be 0.

2D Rf

Reports the 2D Rf values as a %.

Region Centroid (X,Y)

Reports the coordinates (in mm) of the centroid of the counts in each region.

Region Area

Reports the area of each region in mm².

Region Shape

Reports the shape of each region: ellipse, rectangle, or irregular.

**Print Image of Template
Area**

Prints the area of the image within the rectangle that encompasses the group of regions on the report (reported only when you print the report). The template overlay is printed on the image if it is shown on the screen.

OK

Generates the report.

CANCEL

Abandons the operation and closes the window.

The Region Template Report

The Region Template Report shown in Figure 10-5 has a header on the first three lines. Each header provides identifying information about the image.

The first header contains:

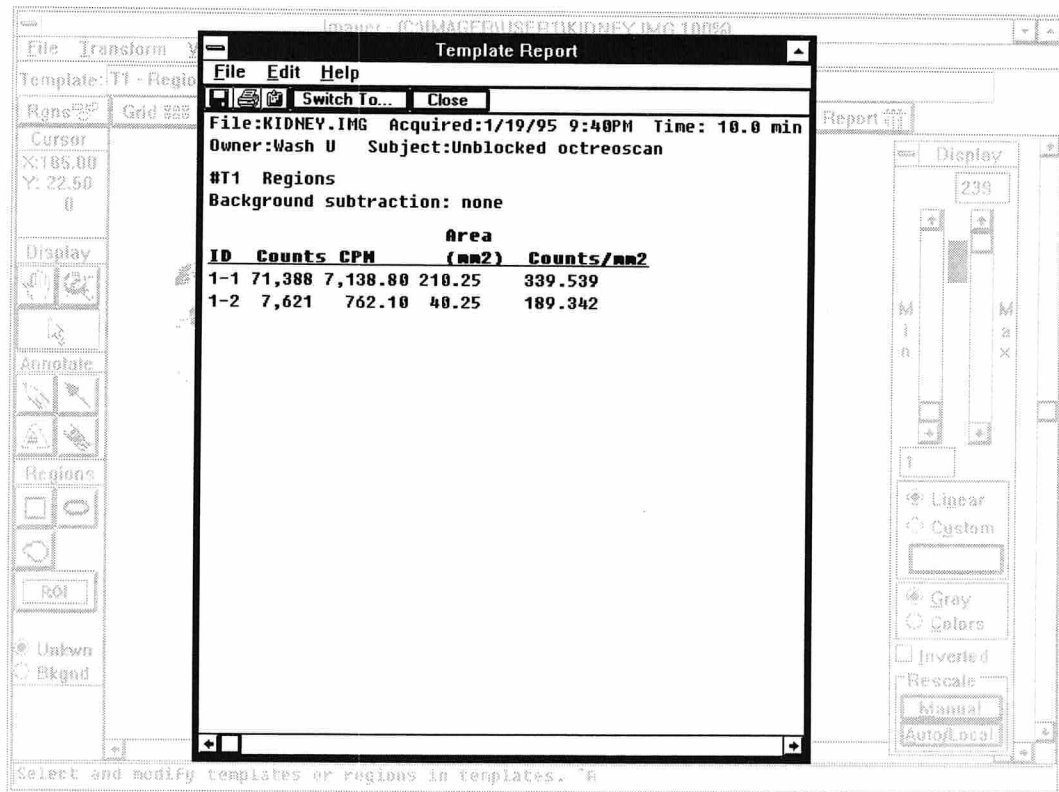
1. **File:** The filename of the image.
2. **Acquired:** The date and time the image was acquired.
3. **Time:** The total elapsed time of the acquisition. The serial number of the instrument's detector may also be displayed.
4. **Owner/Subject:** The initials of the person responsible for the acquisition. The subject of the image can also be displayed.

The second header contains:

5. **Template Number:** The number of the template that is being reported.
6. **Background Subtraction:** Indicates the method of background subtraction selected at the **Region Group Report Options** window.

The third header labels the columns for the remaining options selected at the **Region Group Report Options** window:

7. **ID:** The ID of the region within the selected region group.
8. **Counts:** The sum of all counts in all the pixels within the region.
9. **CPM:** The total counts in the region divided by the total counting time.
10. **Area (mm²):** The total area of the region in mm².
11. **Counts/mm²:** The total counts in the region divided by the area of the region in mm².

**Figure 10-5. Region Template Report.**

Reporting Lanes

Samples organized in lanes or tracks can be analyzed with a lane template. Examples include one dimensional electrophoresis or thin layer chromatography samples. Each template can be comprised of one or many lanes. Using lanes, you can quantitate the activity in bands or spots and determine molecular weights.

Templates include customized report options. These options can be saved for use with other samples.

You can report the quantitative results obtained from a selected lane template.

Using the Lane Group Report Options Window

The **Lane Group Report Options** window (Figure 10-6) enables you to report the quantitative results obtained from a lane template.

Using this window, you can select as few or as many report options as desired. When you select all of the options, you may need to scroll the ensuing reports window horizontally to view all of the information.

You can also select the lanes to be reported by highlighting the lane numbers in the Lane drop-down menu of the window. If the entire lane template is the selected template object when the window is opened, all the lanes will be highlighted. If an individual lane is the selected template object, the lane will be highlighted. Select a single lane number with the left mouse button, or drag the mouse over a range of numbers to select the range. Select **ALL** to highlight all the lanes. Use the **<Ctrl>** key to select any combination.

A lane template must be selected to report results.

To display the **Lane Group Report Options** window:

- ☐ Select **Report Template** from the **Analysis** menu.

Or,

- ☐ Select **REPORT** from the template bar.

 **Note:** *If more than one template is on the image, the **Template Report** window is displayed. See the section *Using the Template Report Window*, (page GS103).*

The report options at the **Lane Group Report Options** window are described below.

Select **OK** to close the window and accept the report options for the selected lanes.

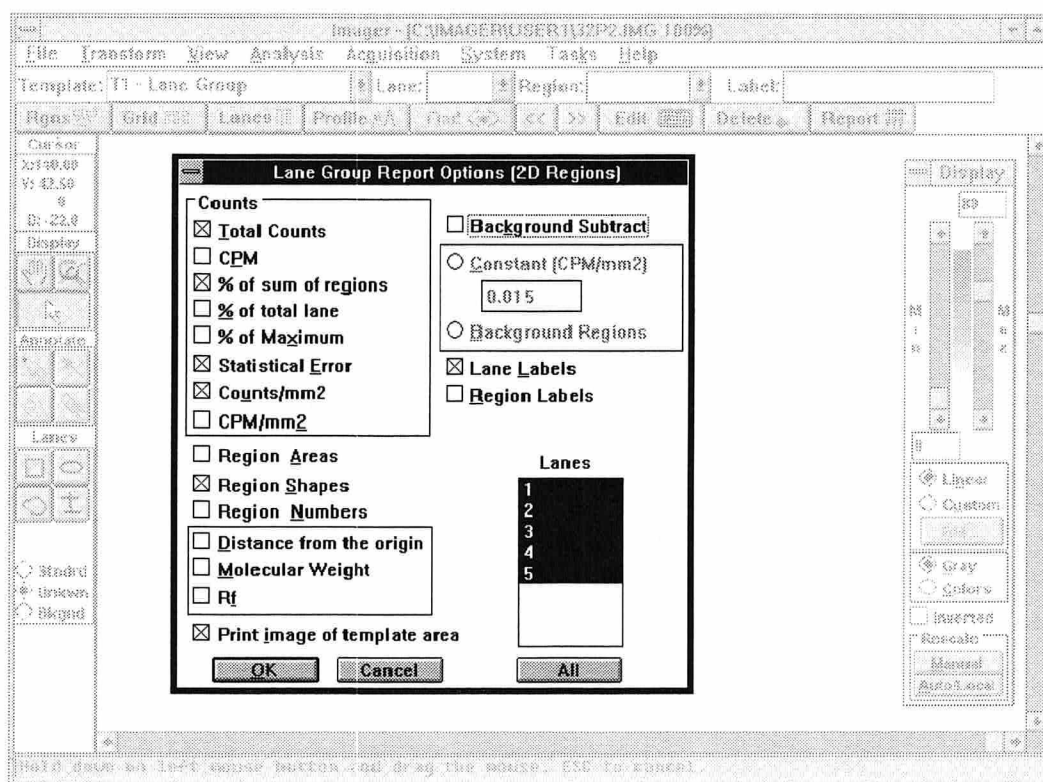


Figure 10-6. Lane Group Report Options Window.

SELECTION**DESCRIPTION OF USE****Counts:
Total Counts**

Reports the sum of the counts in the pixels within the regions.

Counts: CPM

Divides the total counts in the regions by the counting time.

% of Sum of Regions

Reports the counts in each region in a lane as a fraction of the sum of the activity in all regions in the lane.

% of Total Lane

Reports the counts as a fraction of the total counts in the lane.

% of Maximum

Reports the counts in each region in the lane as a fraction of the total counts of the maximum region in the lane.

Statistical Error

Reports the statistical counting error in percent.

Counts/mm²

Divides the total counts in the regions by the total area covered by the region.

CPM/mm²

Divides the CPM of the regions by the total area covered by the region.

Region Areas

Reports the area of each region in millimeters².

Region Shapes

Reports the shape of each region: ellipse, rectangle, irregular, profile, or band.

<u>SELECTION</u>	<u>DESCRIPTION OF USE</u>
Region Numbers	Reports the region number as listed in the template bar or in the region tags on the image. Regions are numbered in the order they are created.
Lane Labels	Reports the labels (if any) entered to identify the lanes as listed in the template bar.
Region Labels	Reports the labels (if any) entered to identify the regions. The labels are listed in the template bar or in the region tags on the image.
Distance From the Origin	Reports the distance from the origin line of the centroid of the counts of the regions. The distance is reported in millimeters.
Molecular Weight	Reports the molecular weights of the unknown regions. This option is grayed out if molecular weight standards have not been defined.
Rf:	Reports the Rf as a value between 0 and 1, with 0=0% and 1=100%. The Rf value is the distance from the line of origin to the top-most spot in the image. It is also the distance from the bottom of the template to the bottom-most spot in the image (within the template).
Print Image of Template Area	Prints the area of the image within the rectangle that encompasses the template on the report (reported only when you print the report). The template overlay is printed on the image if it is shown on the screen.

SELECTION**DESCRIPTION OF USE****Lanes**

Select the lanes to be reported by highlighting the lane numbers in the box. If the entire lane template is the selected template object when the window is opened, all the lanes will be highlighted. If an individual lane is the selected template object, the lane will be highlighted.

Select a single lane number with the left mouse button, or drag the mouse over a range of numbers to select the range. Select **ALL** to highlight all the lanes.

Background Subtract

Select this option and then one of the highlighted background subtracting methods below. With both methods the background value in CPM/mm² will be multiplied by the area of each region. This product will be subtracted from the CPM in the region.

**Background Subtract:
Constant (CPM/mm²)**

Select this option and enter a numerical value in CPM/mm².

**Background Subtract:
Background Regions**

Select this option to determine the background from a region or regions defined as background within each lane. The background value will be the sum of the CPM in the background regions divided by the total area of the regions. If no background regions exist, the amount of background subtraction will be 0.

ALL

Selects all of the lanes in the template.

OK

Generates the report.

CANCEL

Aborts the operation and closes the window.

The Lane Template Report

The Lane Template Report shown in (Figure 10-7) has a header on the first three lines. Each header provides identifying information about the image.

The first header contains:

1. **File:** The filename of the image.
2. **Acquired:** The date and time the image was acquired.
3. **Time:** The total elapsed time of the acquisition.

The second header contains:

4. **Template Number:** The number of the template that is being reported.
5. **Background Subtraction:** Indicates the method of background subtraction selected at the **Lane Group Report Options** window.

The third header labels the columns for the remaining options selected at the **Lane Group Report Options** window:

6. **Shape:** Reports the shape of each region: ellipse, rectangle, irregular, profile, or band.
7. **Error%:** Reports the statistical counting error in percent.
8. **Counts Total:** Reports the sum of all counts in all pixels within the regions.
9. **Counts % Total Regions:** Reports the counts in each region in a lane as a fraction of the sum of all the activity in all regions in the lane.
10. **Counts/mm2:** Divides the total counts in the regions by the total area covered by the region.

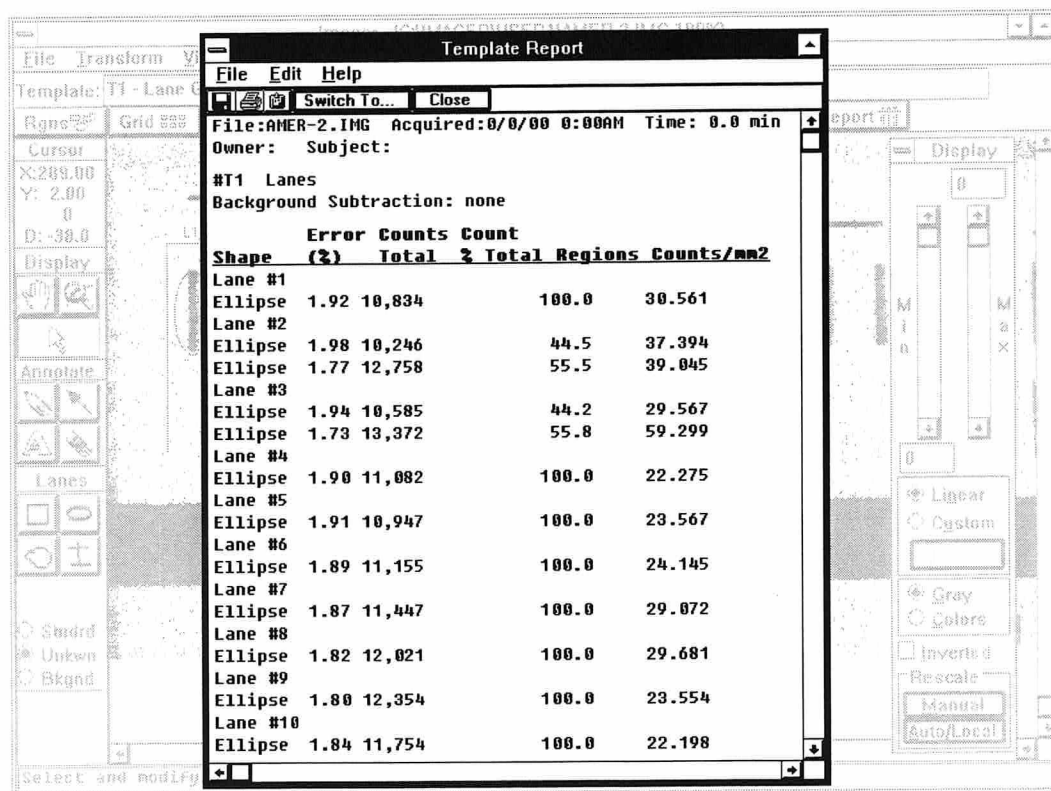


Figure 10-7. Lane Template Report.

Using the Template Report Window

When more than one template is displayed on the screen and you select to generate a report, the names of all templates included on the current image are displayed in the **Template Report** window (Figure 10-8).

The currently selected template is highlighted in the list. Highlight one or all the templates for reporting.

The selections at the window are described below.

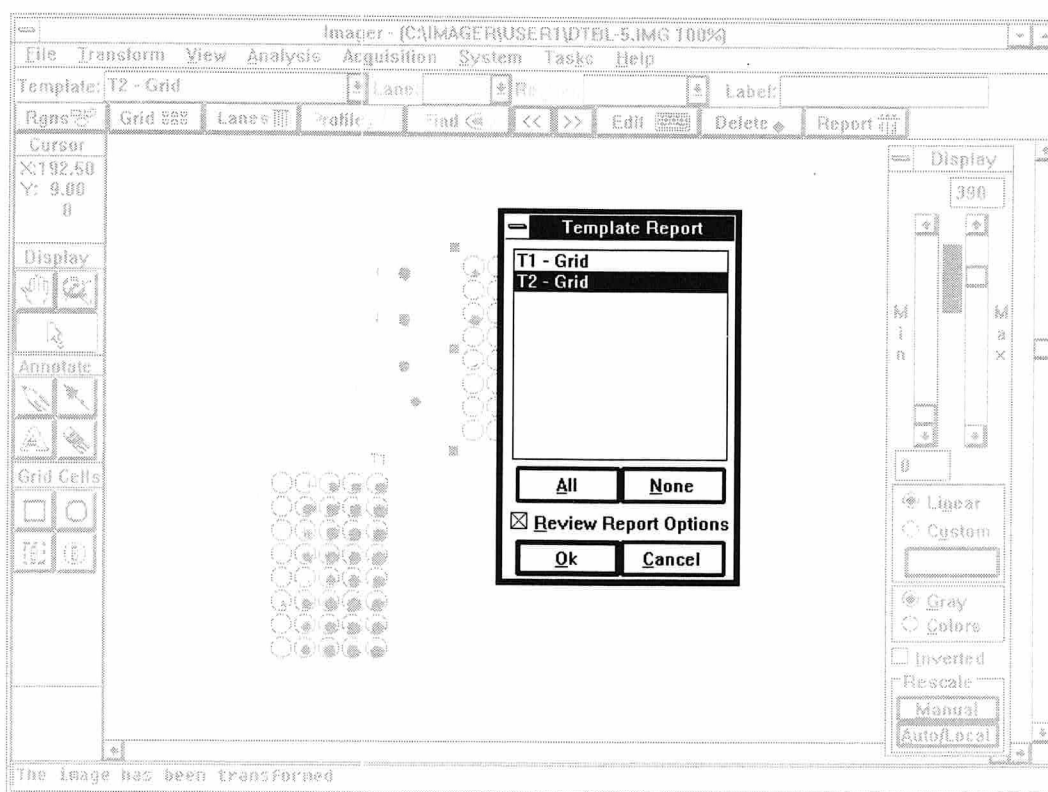


Figure 10-8. Template Report Window.

SELECTION**DESCRIPTION OF USE****ALL**

Selects all the templates listed in the window. Each report is generated successively in the order listed.

NONE

Deselects previously selected templates in the list.

OK

Generates the template reports.

CANCEL

Ignores the operation and closes the window.

Review Report Options

Displays the **Report Options** windows for all the selected templates before the report is created. Changes can be made to the lists of report options. The default for this option is always whatever was selected last.

Selecting One Template

To select one template in the list:

- ☐ Click on the template.

The system highlights the template.

Selecting Templates in Sequence

To select several templates in sequence:

- ☐ Click on the first template in the sequential list.

The system highlights the template.

- ☐ Hold down the **<Shift>** key and select the last template in the group.

The system highlights the entire group.

Selecting Templates Out of Sequence

To select a group of templates that are not in sequence in the list:

- ☐ Click on the first template you want to select.

The system highlights the template.

- ☐ Hold down the <Ctrl> key and click on each item you want to add to the selected group.

The system highlights each selected item.

Selecting all Templates

To select all of the templates listed in the window:

- ☐ Select **All**.

Or:

- ☐ Click on the first template in the list.
- ☐ Hold down the <Ctrl> key and drag the pointer down the entire list.

The system highlights the entire list.

When the Template(s) Have Been Selected

Once you make your selections:

- ☐ Select **OK**.

When you have selected "Review Report Options", the appropriate report options window is displayed for each selected template. This option enables you to make last minute changes to report options before generating a report.

When "Review Report Options" is not selected, each template report is displayed in succession in one window.